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Anti-ulcer activities of ethanolic extract of *Enantia chlorantha* stem bark in rodents

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Abstract

Male adult albino rats (150 – 180 g) were used for the experiment. They were randomized into six (6) groups of six (6) animals each. Food was withdrawn 24 hr and water 2 hr before the commencement of the experiment. Three models were used for this experiment (indomethacin induced ulcer, ethanol and reserpine induced ulcer). Groups 2-4 were pretreated with the extract at doses of 32.40mg/kg, 64.80 mg/kg and 96.20 mg/kg. Cimetidine was used as the standard drug. There was a progressive decline in ulcer index of the rats pre-treated with the extract. The decline was dose-dependent and statistically significant ($p < 0.05-0.001$) relative to control. The extract also showed a progressive preventive ratio. This effect was dose-dependent. The above results provide support for the traditional use of *Enantia chlorantha* stem bark in treating gastric conditions in Akwa Ibom state Nigeria.

Keywords: *Enantia chlorantha*, stem bark, rodents, ethanol extract, ulcers.

1. Introduction

Gastric ulcerations are some of the most common problems of the gastro intestinal tract. There is associated high mortality and sometimes morbidity from bleeding ulcers in the population. They are formed when there is a disruption of the balance between gastric acid secretions (acid, pepsin and helicobacter pylori) and the gastro protective mucus layer which includes bicarbonate, mucin and prostaglandins. Traditionally, the people of Akwa Ibom state in Nigeria have used herbal remedies to treat gastric symptoms for ages. *Enantia chlorantha* is a fair sized ornamental forest tree that can reach heights of 30m. It grows in dense shade and may be recognized by its bright yellow slash and conspicuous black fruits^[1]. It is located in the West African region and extends from southern Nigeria to Gabon, Zaire and Angola. It is commonly called African white wood, Moambe Jaune and Annikia chlorantha. Locally, the name varies from place to place^[2]. The Ibibios of Akwa Ibom call it Uno eto, the Yoruba's call it Osupupa or dokita Igbo. The Edo people refer to it as Erenbav bogo while Ikale and Boki tribes refer to it as Osumolu and Kakerim respectively. The family is Annonaceae and the specie is chlorantha. There are over 40 different species that grow in Nigeria as is seen by the various names that it is called and the varied uses to which it is applied. *Enantia chlorantha* is used to treat a wide variety of conditions using its roots, stem bark, fruit and leaves. In Akwa Ibom it is used to treat malaria fever, typhoid fever, jaundice dysentery, wounds, infections high blood pressure and many other related illnesses. It has been used also for anti-viral, anti-candidal and for gastroenteritis^{[3], [4]}. A decoction of the stem bark in illicit gin is usually taken to relieve painful and swollen joints, fever, headache and toothache. Literature search show that the plant is being used by many tribes as a herbal remedy for various ailments including stomach ulcer. Investigators have examined different aspects of the plant. The models they have employed have revealed some significant level of activity in various areas. We aim by this work to use other models in the hope that the mechanism of action of the plant may be inferred and to test the veracity of the local claims

2. Materials and Methods

2.1 Plant material

The plant *Enantia chlorantha* was collected in January 2012 in Uyo the capital city of Akwa Ibom State, Nigeria. It was identified and authenticated by a Taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Akwa Ibom state Nigeria. A voucher specimen (voucher number UUH 018/13) was deposited with the herbarium of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo.

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2.2 Extraction

The plant stem bark was washed and partially air dried for about 2 weeks at ambient temperature ($25^{\circ}\text{C} \pm 1^{\circ}\text{C}$). It was then ground to powder using pestle and mortar. The pulverized sample was divided into two parts. One part was cold macerated in 70 % ethanol at room temperature for 72 hr and then filtered. The filtrate was dried in a rotary evaporator at 40°C . This extract is referred to as crude. The other part was successively and gradually macerated for 72 hr at room temperature in the following solvents: n-hexane, chloroform, ethyl acetate, methanol and water to obtain different fractions. The crude extract and the fractions were stored in the freezer at -4°C until required.

2.3 Acute toxicity study of the extract

The method of Lorke (1983) was used to determine the LD_{50} of the extract in Swiss albino mice. The extract was administered to three groups of mice containing three (3) mice each at a dose range of 100-1000 mg/kg, (i.p). The animals were observed for physical signs of toxicity and the number deaths in each group within 24 hr were recorded. The animals were fasted for 24 hr prior to the experiment but allowed water *ad libitum*. The LD_{50} was calculated as the geometric mean of the maximum dose producing 0 % mortality (A) and the minimum dose producing 100 % mortality (B). $\text{LD}_{50} = \sqrt{AB}$

2.4 Phytochemical screening

The phytochemical screening of the extract was carried out according to the methods of [5-7]. The following bioactive compounds were screened for their presence: saponins, tannins, and alkaloids. Others were flavonoids, anthraquinones, cardiac glycosides and reducing sugars.

2.5 Animal stock

Swiss albino wistar rats weighing 150-180 g of both sexes were used for the experiments. They were obtained from the Department Of Pharmacology Animal House In The University Of Uyo, Uyo. The animals were housed in standard plastic cages at room temperature and moisture under naturally illuminated environment of 12:12 h dark/light cycle. They were fed with pelleted feeds (Bendel Feeds) and allowed water *ad libitum*.

2.6 Assessment of anti-ulcer activities

2.6.1 Effect of the extract on indomethacin-induced gastric ulceration in rats

Male adult albino rats (150 – 180 g) were used for the experiment. They were randomized into six (6) groups of six (6) animals each. Food was withdrawn 24 hr and water 2 hr before the commencement of the experiment [8]. Group 1 received only indomethacin (60 mg/kg p.o. dissolved in 5 % Na_2CO_3), groups 2-4 were pre-treated with the extract (32.40, 64.80 and 96.20 mg/kg, p.o.) respectively. Group 5 received cimetidine (100 mg/kg dissolved in 10% Tween 80), while group 6 received cimetidine (100 mg/kg p.o.), 10 min later, the extract (64.80 mg/kg p.o) was administered. One hour later, groups 2-6 were administered with indomethacin. The drugs were administered intragastrically with the aid of an orogastric cannula. Four hours after indomethacin administration, animals were sacrificed by ether anesthesia overdose. The stomachs were removed and opened along the greater curvature. The tissues were fixed with 10 % formaldehyde in saline. Macroscopic examination was carried out with a hand lens and the presence of ulcer lesion was scored [9]. Ulcer index (UI), Preventive ratio (PR) and degree

of ulceration (DU) of each of the groups pre-treated with the extract were calculated using standard methods [10-12].

A score of zero (0) was given to cases with no visible lesions while 1 was given to one ulcer and generalized edema. A score of 2 was allotted to lesions that had at least 2 ulcers measuring approximately 2mm. ulcers measuring 1-4mm found along 80% of the gastric fold received a score of 3 and those lesions that followed 80% of the fold were given a score of 4 while ulcers found along the whole length of the intestine examined were allotted a the maximum score of 5.

2.6.2 Effect of the extract on ethanol-induced gastric ulceration in rats

The procedure used was similar to that used in indomethacin induced ulceration. The rats were randomly assigned to six (6) groups of six (6) animals each. Food was withdrawn 24 hr and water 2 hr before the commencement of the experiment. Group I received 1 ml of ethanol (control), Groups 2-4 were pretreated extract (32.40, 64.80 and 96.20 mg/kg, p.o.) respectively. Group 5 received propranolol (40 mg/kg p.o dissolved in distilled water), while group 6 received propranolol and the extract (64.80 mg/kg p.o.) administered ten (10) minutes later. One hour later groups 2-6 were administered with 1 ml of 99.5 % ethanol to induce ulcer. The drugs were administered intra-gastrically via the aid of an orogastric cannula. Four hours (4 hr) after ethanol administration, animals were sacrificed by cervical dislocation. The stomachs were removed and opened along the greater curvature. The tissues were fixed with 10 % formaldehyde in saline. Macroscopic examination was carried out with a hand lens and the presence of ulcer lesion was scored using standard methods [13, 12].

The highest score of 5 was given to multiple ulcers found along the entire length off the gastric fold that was examined. A score of 4 was allocated to lesions found along 80% of the gastric fold. Ulcers that were 1-4 mm in diameter found along 80% of the length of gastric fold were awarded a score of 3. A score of 2 was given to ulcers that were at least 2 in number with a minimum diameter of 2mm. The presence of 1 ulcer and generalized erythema received a score of 1. When there were no detectable ulcers, then a score of 0 (zero) was allotted.

2.6.3 Effect of the extract on reserpine-induced gastric ulceration in rats

Adult albino male rats (120 – 170 g) were used for the experiment. They were randomized into six (6) groups of six (6) rats each. Food was withdrawn 24 hr and water 2 hr before the commencement of experiment (Alphin and Ward, 1967). [8] Group 1 (control) received only reserpine (Sigma, 8 mg/kg, i.p dissolved in 10 % Tween 80); Groups 2-4 were pretreated with the extract (32.40, 64.80 and 96.20 mg/kg, p.o.) respectively. Group 5 received cimetidine (100 mg/kg dissolved in 10 % Tween 80), while group 6 received cimetidine (100 mg/kg p.o.), 10 min later, the extract (64.80 mg/kg p.o) was administered. One hour (1 hr) later, group's 2-6 were administered with reserpine, 8 mg/kg i.p dissolved in 10 % Tween 80 [14]. Eighteen (18) hr later animals were sacrificed by cervical dislocation. The stomachs were removed and opened along the greater curvature. The tissues were fixed with 10 % formaldehyde in saline. Macroscopic examination was carried out with a hand lens and the presence of ulcer lesion was scored [9]. Ulcer index (UI), preventive ratio (PR) and degree of ulceration (DU) of each of the groups pretreated with extract was calculated using standard method [10, 13] as shown above.

2.7 Statistical analysis

Results were expressed as multiple comparisons of mean \pm SEM. Significance was determined using One-way Analysis of Variance (ANOVA) followed by Turkey-Kramer multiple comparison post test. A probability level of less than 5 % was considered significant.

Table 1: Effect of extract on indomethacin-induced ulcer

| Treatment | Dose (mg/kg) | Ulcer Indices | Preventive Ratio |
|------------------------|--------------|-------------------------------|------------------|
| Control (indomethacin) | 60 | 15.00 \pm 0.00 | - |
| Extract | 32.40 | 11.65 \pm 1.65 ^a | 22.33 |
| Extract | 64.80 | 8.30 \pm 0.33 ^b | 44.66 |
| Extract | 96.20 | 3.30 \pm 1.08 ^c | 78.00 |
| Cimetidine | 100.00 | 0.80 \pm 0.16 ^c | 94.66 |
| Extract +Cimetidine | 64.80 + 100 | 0.83 \pm 0.16 ^c | 94.46 |

Data were expressed as mean \pm SEM.

Significant at ^ap<0.05, ^bp<0.01, ^cp< 0.001 when compared to control (n = 6).

2.2 Effect of Extract on Ethanol-induced Ulcer in Rats

Table 2 shows the results of anti-ulcer activity of the extract against ethanol-induced ulceration in rats. Pre-treatment of rats with the extract resulted in a dose-dependent decrease in the ulcer index. This decrease was statistically significant (p<0.001) compared to control. The extract also showed a progressive preventive ratio on ulcer induced by ethanol.

Table 2: Effect of extract on ethanol-induced ulcer

| Treatment | Dose (mg/kg) | Ulcer Indices | Preventive Ratio |
|----------------------|---------------|------------------------------|------------------|
| Control (ethanol) | - | 5.00 \pm 0.00 | - |
| Extract | 32.40 | 4.33 \pm 0.33 ^b | 13.40 |
| Extract | 64.80 | 2.66 \pm 0.33 ^b | 46.80 |
| Extract | 96.20 | 0.66 \pm 0.33 ^c | 86.80 |
| Propranolol | 40.00 | 0.33 \pm 0.33 ^c | 93.40 |
| Extract +Propranolol | 64.80 + 40.00 | 2.33 \pm 0.33 ^c | 53.40 |

Data were expressed as mean \pm SEM.

Significant at ^bp< 0.01, ^cp< 0.001, when compared to control (n = 6).

3.3 Effect of Extract on Reserpine-induced Ulcer in Rats

The effect of extract pretreatment on reserpine-induced gastric ulceration in rats is as shown in Table 3. The extract significantly (p<0.001) reduced the ulcer indices relative to control in a dose-dependent manner. The extract demonstrated a progressive increase in preventive ratio of ulcer induced by reserpine. However, the decrease was lower than that of the standard drug, cimetidine (100 mg/kg).

Table 3: Effect of extract on reserpine-induced ulceration in rats

| Treatment | Dose (mg/kg) | Ulcer Index | Preventive Ratio |
|----------------------|----------------|------------------------------|------------------|
| Control (ethanol) | - | 5.00 \pm 0.00 | - |
| Extract | 32.40 | 3.33 \pm 0.33 ^b | 33.40 |
| Extract | 64.80 | 2.66 \pm 0.33 ^b | 46.80 |
| Extract | 96.20 | 1.00 \pm 0.00 ^c | 80.00 |
| Cimetidine | 100.00 | 0.33 \pm 0.33 ^c | 93.40 |
| Extract + cimetidine | 64.80 + 100.00 | 1.66 \pm 0.33 ^c | 66.80 |

Data were expressed as mean \pm SEM.

Significant at ^bp< 0.01, ^cp< 0.001 when compared to control (n = 6).

4. Discussions

The anti-ulcer activity of the extract was evaluated using indomethacin, ethanol and reserpine-induced ulcer models in

3 Results

3.1 Effect of extract on indomethacin-induced ulcer in rats

The results of anti-ulcer activity of the extract on indomethacin-induced ulceration in rats is shown in Table 1. There was a progressive decline in ulcer index of the rats pre-treated with the extract. The decline was dose-dependent and statistically significant (p<0.05–0.001) relative to control. The extract also showed a progressive preventive ratio. This effect was dose-dependent.

rats. Indomethacin causes ulcer in an empty stomach [15] and the glandular (mucosal) part of the stomach is mostly affected [16-9] by inhibiting prostaglandin synthetase through the cyclooxygenase pathway [17]. Prostaglandins protect the stomach from injury by stimulating the secretion of bicarbonate and mucus. This maintains mucosal blood flow and regulates mucosal turn over and repair [18, 19]. When prostaglandin synthesis is suppressed by indomethacin it results in increase susceptibility of the stomach to mucosal injury and gastroduodenal ulceration [19]. The extract was seen to significantly reduce mucosal damage in the indomethacin-induced ulcer model. This suggests possible mobilization and involvement of prostaglandin in the anti-ulcer effect of the extract [12].

Ethanol has been reported to cause gastrointestinal tract disturbances such as increase gastric secretion, changes in the permeability of gastric mucosa, gastric mucus depletion, damage to the mucosal wall and increase free radical production [20]. The release of superoxide anions and hydroperoxy free radicals during metabolism of ethanol as oxygen-derived free radicals may be responsible for these effects. They have been implicated in the mechanism of acute and chronic ulceration of the gastric mucosa [21]. In this study, the extract significantly reduced ethanol-induced ulcers. This cyto-protective effect of the extract may be due to its antioxidant effects. Ethanol is also reported to cause gastric mucosal damage by stimulating the formation of leukotriene C₄ (LTC₄) [22]. The gastro-protective effect of the extract may in part be due to its ability to suppress lipoxygenase activity.

The mechanism at which reserpine produces ulceration is not completely understood. Although there are suggestions as to what could be responsible for this action, they include Reserpine-induced gastric ulceration, which has been attributed to vagotonic hypermotility and degranulation of gastric mast cells. This results in increase in gastric acid secretion which is believed to be cholinergically mediated [23], [24]. Several factors may be responsible for the ulceration potentials of reserpine. They include mobilization of superoxide and hydroxyl free radicals as is found in ethanol, [25, 26] inhibition of mucous release and stimulation of surface mucous breakdown which occurs through β adrenoceptor stimulation. Accordingly, the anti-ulcer activity of the extract against reserpine-induced ulceration may also be due to its inhibition of histamine, anti-cholinergic and anti-secretory effects. The extract has ability to inhibit oxygen-derived free

radicals formation in rat gastric mucosa and stimulate endogenous prostaglandin synthesis which may be responsible for this effect^[27]. Phytochemical analysis of the extract revealed the presence of saponins and tannins. Saponins, especially triterpenes type have been implicated in antiulcer activity. This effect is mediated by the formation of protective mucus on the gastric mucosa and protection of the mucosal cell wall from acid effects by selectively inhibiting PGF_{2α}^[28, 29]. Tannins are known to be astringents and may have precipitated microproteins on the ulcer site. This forms an impervious protective pellicle layer over the lining. This has the effect of preventing the absorption of toxic substances and also affords protection against proteolytic enzymes^[30].

Conflict of interest statement

We declare that we have no conflict of interest in the pursuit of this work.

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